

# RNA Interference for Tumor Therapy

Wei Xia and Jing Ni

*Department of Nuclear Medicine, Shanghai Seventh People's Hospital, Shanghai,  
P.R. China*

## 1. Introduction

The most recent investigation from the World Health Organization's International Agency for Research on Cancer (WHO/IARC) indicates that the incidence of malignant tumors is rising throughout the world. In 2008, 7.6 million people died of malignant tumors and 64% of them occurred in developing countries (Jemal *et al*, 2011). Particularly, the status on the treatment of tumors is extremely harsh in China since both incidence and mortality of malignant tumors in China is drastically rising in the past two decades (Zhang *et al*, 2008). It is expected that this increasing tendency will continue in China in the next two decades (Xv&Dong,2003). There is no doubt that tumor therapy will be for long term a hot topic in the field of life and medicine in the future.

The extensive understanding of molecular mechanisms underlying tumor development and progression provides more deep insights that tumor is a genetic disease. It appears that the correction of defective genes would likely be a new approach for the treatment of tumors (Wang *et al*, 2010). RNA interference (RNAi) is a double-stranded RNA-induced gene silencing process, by which RNA can specifically silence complementary mRNA in the cells. Fire *et al*. for the first time discovered this phenomenon in the cells of rod-shaped nematomorpha (Fire *et al*, 1998), for which he was awarded the Nobel Prize in Physiology or Medicine in 2006. Further studies found that RNAi exists in almost all multi-cellular organisms and plays an important role in defense against double-stranded RNA virus (Hannon,2002). Also, RNAi-based tumor therapy in animal models has demonstrated that RNAi is a powerful means for tumor therapy (López-Fraga *et al*, 2009; Davis *et al*, 2010; Koldehoff& Elmaagacli,2009; Maples *et al*, 2009).

This chapter aims to review current progress in RNAi for tumor therapy. The mechanisms underlying RNAi-induced gene silencing pathway were briefly described in the first section. Next, the application of RNAi targeting different tumor-related genes was summarized in detail. Finally, recent achievement of RNAi-based tumor therapy in clinical trials was also reviewed.

## 2. Mechanisms of RNAi

The RNAi phenomenon was first found in antisense hybridization of antisense RNA and mRNA. However, further studies showed that the sense strand could also result in reduced gene expression (Guo& Kempfues,1995). Subsequent studies demonstrated that each strand

of dsRNA can induce RNAi, but only few types of dsRNA can lead to potent interference effect (Fire *et al*, 1998). The molecules that can trigger RNAi include small interference RNA (siRNA), microRNA (miRNA) and small hairpin RNA (shRNA). The mechanisms of RNAi triggered by these molecules are not exactly the same.

## 2.1 Mechanisms of siRNA

SiRNA is a class of dsRNA molecules with 20-25 nucleotides in length. When dsRNA enters cells, it can be lysed into siRNA by Dicer enzyme. On the other hand, dsRNA proliferates in the presence of RNA-directed RNA polymerase (RdRP) and is then lysed in the presence of Dicer enzyme. Double-stranded siRNA is processed by Dicer into single-stranded siRNA, which can form a complex via binding with some related proteins. This complex can bind simultaneously with complementary mRNA, thereby inducing mRNA lysis.

SiRNA-induced RNAi is initiated from a cleavage program. Long dsRNA is first cleaved by Dicer into siRNAs in cytoplasm. The Dicer contains an N-terminal helicase domain, a PAZ (Piwi/Argonaute/Zwille) RNA domain, two RNase III domains, and a dsRNA binding domain (dsRBD) (Bernstein *et al*, 2001). The RNA helicase domain sequence is highly stable and N-terminal and C-terminal are core components. The N-terminal has an ATP-binding and hydrolysis subunit, and the C-terminal contains a RNA-binding subunit. With these subunits, the RNA helicase domain can play a helicase function by making use of the energy provided by ATP (Ma *et al*, 2004). The PAZ domain of Dicer can specifically recognize and bind with the 5' phosphate bond with two nucleotide protrusions in the dsRNA3' end (Cordin *et al*, 2006). There is a long  $\alpha$ -helix between the PAZ domain and the RNase III domain, and this helix is encircled by a conserved N-terminal protein, forming a platform domain consisting of an anti-parallel  $\beta$ -sheet and three  $\alpha$ -helices, and at both ends of the platform domain are hinge1 and hinge 2 as a polypeptide chain to hinge with the PAZ domain and the RNase III domain respectively. Of them, hinge1 contains a Pro-266 kink, which enables the PAZ domain to move a 5Å distance. Unlike hinge 1, hinge 2 does not have the link, but it can enable the RNase III domain to make a 5° rotation along the long axis of the Dicer molecule. Therefore, Dicer is able to adapt to substrates of different shapes by regulating the platform, hinge 1 and hinge 2 for the purpose of cleaving different substrates (Macrae *et al*, 2006). Each RNase III domain possesses two active centers, and each center has the activity of cleaving the phosphodiester bond, and can cleave dsRNA into small RNA with two nucleotide protrusions in the dsRNA3' end, which is the typical characteristic of the RNase III product (Collins&Cheng,2005). Although Dicer contains two RNase III domains and four active cleavage centers, each RNase III domain provides only one active center for cleavage. The distance between the active centers of the two RNase III domains is 65Å apart, equaling 25 bp RNA in length. This structural characteristic ensures Dicer to specifically produce small RNA of 21~25 bp in length, and enables it to change the length of the cleaved product by mirco-regulating the distance between the two RNase III domains (Błaszczyk *et al*, 2001). In the C-terminal of Dicer is the dsRBD that contains about 70 amino acids existing in the form of  $\alpha\beta\beta\alpha$ , of which the  $\alpha$ -helix is close to one side of  $\beta$ -sheet in a reverse and parallel form (Hallegger *et al*, 2006). dsRBD and dsRNA interact with each other across a width of 16 bp, which is just the distance between the two small adjacent grooves and the large groove on one side of  $\alpha$ -dsRNA(Wu *et al*, 2004). This interacting region can be further divided into three small regions. Region 1: the four 2'-OH at the first

small groove of dsRBD N-terminal  $\alpha$ -helix connect with five nucleotides of RNA. Region 2: a stem loop between  $\beta 1$  and  $\beta 2$  of dsRBD connects with 2-5 nucleotides of RNA in the second small groove through 2'-OH. Region 3: the  $\alpha$ -helix at the C-terminal of dsRBD travels across the large groove of RNA and connects with the phosphodiester bond in RNA (Ryter & Schultz, 1998).

The long dsRNA that enters cells is processed by Dicer into double-stranded siRNA, and then bind with Dicer, trans-activator response region RNA-binding protein (TRBP), and protein kinase R (PKR)-activating protein (PACT) to form a RNA-induced silencing complex (RISC) (Hammond *et al*, 2000).

RNAi induced by completely processed single-stranded siRNA (mostly exogenous) can be directly installed into RISC without the participation of TRBP/PACT-Dicer complex. However, in other RNAi processes, RNAi cannot be installed into RISC without the participation of TRBP/PACT-Dicer complex. Through TRBP/PACT, siRNA-TRBP/PACT-Dicer complex binds with Argonaute 2 (Ago2/EIF2C2) to form RISC loading complex (RLC) (Robb & Rana, 2007). RLC initiates disentanglement of siRNA and distinguishes the guide strand and the passenger strand. The guide strand 5'-end has the property of a low thermodynamic stability and can be bound into RISC by priority (Tomari *et al*, 2004). The passenger strand is cleaved by Ago 2 and separated from RLC (Matranga *et al*, 2005). The specific sequence for binding between the RISC-mediated RNA guide strand and target mRNA is mainly located in the "seed" or "core" region of 2-8 nucleotides of siRNA 5'-end (Ma *et al*, 2005). mRNA oligonucleotide is cleaved from the middle in the presence of Ago2 and loses the expression function (Schwarz *et al*, 2004; Hammond, 2005).

Ago 2 is the only protein that has the endonuclease function in RLC, mainly consisting of an N-terminal domain, a PAZ domain, a middle domain and a PIWI domain, of which the N-terminal domain, middle domain and PIWI domain form a crescent basin structure, with one "arm" supporting the PAZ domain above the "basin" (Song *et al*, 2004; Yuan *et al*, 2005). This structure forms a groove carrying positive charge. The N-terminal domain and PIWI domain form the small groove, which can bind with nucleic acid. The PAZ domain and the crescent "basin" form the large groove, which can contain double strands and locate the easily broken phosphate bond directly to the catalytic site of RNase H sheet on the PIWI domain. Ago 2 can melt siRNA, forming single-stranded antisense siRNA complementary to target mRNA. The RNase H PIWI domain of Ago 2 is located in the 5'-end, and the PAZ domain can recognize siRNA 3'-end. When siRNA binds with complementary target mRNA, Ago 2 begins digesting mRNA, forming RNA fragments with 5'-carboxylate and 3'-phosphate to degrade mRNA of the endogenous target gene, thus preventing gene expression. During the process of degradation, mRNA comes in between the N-terminal and the PAZ domain and goes out between the PAZ domain and the middle domain. When mRNA moves between the PAZ domain and the "crescent basin", it is cleaved by the active site of the PIWI domain.

RISC can be recycled. When a target mRNA is degraded, RISC can continue to degrade other target mRNA, thus amplifying the PTGS effect of the target gene (Gregory *et al*, 2005) and this amplification can maintain for few days in dividing cells. Obviously, it is advantageous over other therapeutic methods, and is a potent method for specific inhibition of gene expression. In addition, a few studies (Meister *et al*, 2005) also found that RISC also

has some other protein components, including Vasa intronic gene (VIG) protein, DmFXR, Tudor-SN, potential RNA helicase Dmp68 and Gemin3. These proteins are not necessary for the activity of RISC nuclease, but may provide additional functions such as RISC turnover, RISC subcellular location, and degradation after mRNA cleavage.

## 2.2 Mechanisms of miRNA

miRNAs are a class of endogenous non-coding RNA that serves a specific function in eukaryotic organisms, with about 20~25 nucleotides. Mature miRNAs are processed from relatively long primary transcripts through a series of nuclease cutting, and then assembled into the RNA-induced silencing complex to identify target mRNA through base pairing, and instruct the silencing complex to degrade or block the translation of target mRNA depending on the degree of complementation.

miRNA genes are usually transcribed by RNA polymerase II (pol II) in the nucleus, and the primary product is pre-miRNA with a large cap-structure (7MGpppG) and a poly-A tail (AAAAA). Pre-miRNA is treated in stem loop-structured pre-mRNA containing 60-110 nucleotides in the presence of RNase III enzyme Drosha (RNASEN) and its cofactor DGCR8 (a double-strand RNA domain-binding protein)(Tang,2005), and this pre-miRNA is transported from the nucleus to the cytoplasm by RAN-GTP and exportin 5 (EXP 5), and then cleaved by another nuclease Dicer to produce miRNA:miRNA double strands about 22 nucleotides in length, which are quickly introduced into RISC containing Ago2. One of the single-stranded mature miRNA is retained in the complex and expressed in response to regulatory genes in a manner similar to siRNA at the compensatory site of mRNA via base pairing (Meister&Tuschl,2004). Apart from Ago2, Ago1, Ago3 and Ago4 in the argonaute family that have no endonuclease activity also participate in the RNAi process of RISC through regulation on RISC rather than through cleavage (Preall&Sontheimer,2005).

Identification of target mRNA by miRNA requires precise pairing of nucleotides with miRNA seed sequence (Sethupathy *et al*, 2006). If miRNA and mRNA compensate with each other precisely, Ago 2 can cleave mRNA in the same way as it cleaves siRNA (Meister *et al*, 2004). However, there is a convex sequence in the middle of the A-type helical structure from non-precise combination of compensatory miRNA and the target mRNA, which makes mRNA cleavage impossible but can inhibit the expression of target mRNA at the protein translation level. This cleavage-independent mechanism plays the main role in miRNA-induced RNAi (Pillai *et al*, 2007). The miRNA-binding site using this mechanism is usually in 3'-end non-translation region of mRNA. Each miRNA can have multiple target genes, while several miRNAs can also regulate the same gene. This complex regulatory network can regulate expression of multiple genes through one miRNA, or precisely regulate the expression of a particular gene through combination of several miRNAs (Sethupathy *et al*, 2006). About one-third of encoding protein mRNA can be regulated by miRNA(Krek *et al*, 2005).

## 2.3 Mechanisms of shRNA

shRNA contains two short inverted repeat sequences separated by a stem loop in the middle forming a hairpin structure, which is controlled by Pol III promoter and then connects with 5-6 T as the transcription terminator of RNA Pol III. One of the methods of transporting

siRNA *in vivo* is to clone the siRNA sequence into the plasmid as a “short hairpin”. When the hairpin is delivered to the animal body, its sequence is expressed, forming “double-stranded RNA” (shRNA), which is treated by the RNAi pathway.

The first-generation shRNA is decorated and cloned to the viral carrier on the pre-miRNA platform, and transcribed into 50-70bp single-stranded molecules with the unique “stem-loop” structure in the presence of RNA Pol III in the nucleus. When it enters the cytoplasm, its stem-loop structure is cut off by Dicer, forming siRNAs. Finally, it participates in the production of the RISC complex and induces RNAi (Paddison *et al*, 2004; Brummelkamp *et al*, 2003; Berns *et al*, 2004). With further research on miRNA, researchers have re-modeled the well-known structure of human miRNA-mir-30 and successfully developed a carrier-mediated second-generation RNAi trigger shRNAmir using pri-miRNA as the platform. Unlike pri-mir-30, the “stem” of this carrier-mediated shRNAmir has been replaced by other double helixes specific to different target genes. This structural re-design and transformation do not affect normal maturity of mir-30. Compared with the first-generation shRNA, the design of the second-generation shRNAmir makes use of the endogenous Drosha digestion process, thus increasing the specificity of Dicer in identifying Pre-miRNA. In addition, shRNAmir enters the intracellular RNAi pathway than shRNA or siRNA and can be treated by Drosha and Dicer, thus producing more siRNA (Silva *et al*, 2005).

## 2.4 Mechanisms of bifunctional shRNA

Bifunctional shRNA can make use of cleavage-dependent and non cleavage-dependent RNAi (Rao *et al*, 2010). Such shRNA contains two stem loops: one with complete-matching passenger and guide strands, which can combine with cleavage-dependent RISC, and the other with non-matching strands, which can combine with non cleavage-dependent RISC. This shRNA is more efficient than shRNA RNAi with one RISC complex. In experiments directly comparing the same siRNA target RNAi, it was found that bifunctional shRNA was more efficient (the same dosage of shRNA could produce more powerful gene knockout function) and its pharmacokinetics also underwent change (Rao *et al*, 2009, 2010).

## 3. The application of RNAi for silencing of tumor-related genes

RNAi has a standard procedure in intracellular gene knockout and can be used to identify gene functions by inhibiting specific genes and analyzing the change of gene appearance. As such, by analyzing gene functions, the signal pathway of tumor development could be identified. This further provides the feasibility to assess the medical or RNAi therapeutic target (Devi, 2006). In addition, systemic treatment approaches with cancer-related miRNA or miRNA offer opportunities for tumor therapy. There have been a few overexpressed target genes for RNAi study *in vivo*. The section is a retrospective review of these targets.

### 3.1 Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a heparin-binding growth factor specific to vascular endothelial cells, and can induce angiogenesis *in vivo*. VEGF and anti-VEGF coexist in normal tissues and remain in a relatively balanced state. In the presence of tumor growth, multiple carcinogenic factors trigger dramatic increase in VEGF, the amount and function of which are by far greater than those of anti-VEGF, thus causing massive growth

of vessels (mainly blood vessels) and promoting tumor growth and metastasis. In addition, VEGF interferes with and inhibits dendritic cells and blocks antigen presentation of B and T cells, which further induces immune escape of tumors, interferes with the normal immune function of the body, and causes resistance to anti-tumor therapy. He (He *et al*, 2009) used  $\text{CaCO}_3$  as the matrix and form nanoscaled complexed with the plasmid to express siRNA targeting VEGF. In their siRNA transfection against colon cancer cells in vitro, they found that this complex could significantly reduce the expression level of VEGF-C in LoVo cells. In the experiment with transplanted colon cancer in nude mice, the mean tumor size treated by the complexes was decreased by 50% as compared to that in the control group. Metastasis of the cancer cells in lymph nodes and lymphatic ducts was also inhibited.

### 3.2 Vimentin

Cell structures and morphologies are maintained through a series of complex structural proteins, including microtubules, microfilaments and intermediate filaments. Vimentin is a type III intermediate filament. Studies in recent years have demonstrated that vimentin is closely associated with tumor development and metastasis, and participates in adhesion, migration, invasion and cell signal transduction of tumor cells and tumor-related endothelial cells and macrophages. Vimentin possesses a highly dynamic balance between polymerization depolymerization and a very complex phosphorylated form, which serve as the basis for it to participate in tumor metastasis and intercellular actions. Vimentin is found to be homologous to most sequences of miR-17-3p in the miRNA gene cluster 17-92 family. miR-17-3p participates in regulating the expression of vimentin. miR-17-3p is a known tumor-inhibiting gene. It remains at a low level in highly tumorigenic and metastatic cell lines and at a high level in cell lines of low tumorigenesis. Zhang (Zhang *et al*, 2009) used plasmids expressing miR-17-3p to intervene transplanted prostate cancer in mice and found that the tumor size was only 50% of the control group 31 days after the experiment. They also found that vimentin expression was negatively correlated with miR-17-3p expression, and the rate of tumor growth was also negatively correlated with miR-17-3p. Paccione RJ (Paccione *et al*, 2008) used vimentin as the target to perform RNA interference, and found that the ability of cell proliferation, metastasis and invasion in cellRNAi group decreased by 3 fold as compared with the control group. In addition, tumorigenesis of cells treated with vimentin-RNAi decreased significantly, and the size of tumors injected subcutaneously with vimentin-RNAi was 70% smaller than that of the control group.

### 3.3 STAT3

Signal transducer and activator of transcription 3 (STAT3) is a member of the STAT family, participating in signaling pathways closely related to cell proliferation, differentiation and apoptosis. Its abnormal activity may promote cell differentiation and proliferation and inhibit cell apoptosis, causing tumor development and promoting metastasis of tumor cells. It is found to be activated persistently in hepatocellular carcinoma (HCC), breast cancer and esophageal squamous cell carcinoma. Li (Li *et al*, 2009) injected STAT3-target shRNA, negative shRNA and normal saline (NS) into transplanted HCC tumors in nude mice and removed the tumors after observation for a period. It was found that the tumor tissue in STAT3-shRNA, negative shRNA and NS groups was 0.18g, 0.6g and 0.67g respectively. It was also found that the expression of STAT3, phosphorylation of STAT3, and expressions of

VEGF, survivin and MYC were down-regulated, while caspase3 and p53 were up-regulated in STAT3-shRNA group. Huang (Huang *et al*, 2011) studied the effect of RNAi using STAT3 as the target gene on pancreatic cancer, and found that STAT3 RNAi not only inhibited tumor growth but inhibited angiogenesis in the tumors. They also found that VEGF and MMP-2 mRNA and protein levels decreased significantly in SW1990 cells treated with RNAi, indicating that STAT3 plays an important role in the course of tumor development and progression, and can be used as a target for tumor gene therapy.

### 3.4 Human telomerase reverse transcriptase

Human telomerase reverse transcriptase (hTERT) is a rate-limiting enzyme maintaining the activity of telomerase. However, it is found that hTERT not only maintains telomerase but stabilizes cell genomes, promotes cell proliferation, inhibits cell apoptosis, and plays a role in regulating intracellular signaling pathways. A recent study (de Souza Nascimento *et al*, 2006) transfected Hela with shRNA expressing carrier-encoded target shRNA, and found that short telomerase-related hTERT mRNA was reduced dramatically, cell growth was inhibited, and cell apoptosis was accelerated. A similar study (Gandellini *et al*, 2007) showed that transfection of hTERT specific siRNA with lipofectamine 2000 could inhibit the expression of hTERT for a prolonged time, thus affecting the proliferation and tumorigenesis of prostate cancer PC-3 cells.

### 3.5 Survivin

Survivin is a recently discovered tumor-specific inhibitor of the apoptosis protein family. There is no or micro expression in well differentiated tissues, and high expression in embryonic and most human malignant tumor tissues. It inhibits apoptosis of tumor cells, thus promoting cell growth and regulating cell division. In addition, it is closely associated with infiltration and metastasis of tumor cells. Survivin is found expressing in common malignant tumors such as breast cancer, gastric cancer, kidney cancer, melanoma, intestinal cancer, neuroblastoma cancer and ovarian cancer. In their retrospective analysis of tumor treatment, Kanwar (Kanwar *et al*, 2010) demonstrated that survivin-targeted molecular therapy could inhibit the growth of tumor cells. Recently, Chen (Chen&Deng, 2008) injected gastric cancer MGC-803 cells treated with plasmids expressing survivin-siRNA and control-treated plasmids, and those without intervention into nude mice subcutaneously. Four weeks later, the mean tumor volume in the intervention group, control group and non-intervention group was 831mm<sup>3</sup>, 2617mm<sup>3</sup> and 2536mm<sup>3</sup> respectively, indicating that the apoptosis rate in siRNA intervention group was significantly higher than that in the control and non-intervention groups (27.63% vs 2.15% vs 2.31%). In their experiment study with survivin-target RNAi for the treatment of urinary bladder cancer, Seth (Seth *et al*, 2011) reported the similar result and further confirmed that survivin-target RNAi could inhibit tumor cell growth effectively.

### 3.6 ERK-MAPK

ERK-MAPK is activated in many malignant tumors, and plays a primarily important role in cell growth, differentiation and survival. siRNA specific to extracellular signal regulating kinase 1/2 (ERK1/2) could inhibit the proliferation of ovarian cancer cells and

induce their apoptosis and death, but it had no effect on normal ovarian cells (Zeng *et al*, 2005). Super-expression of epithelial growth factor receptor HER2/neu was detected in many breast cancers, but its expression in patients with good prognosis was low. Transfection of HER2/neu siRNA led to an antiproliferative and apoptosis-inducing effect in breast cancer cells with high expression of HER2, and this effect was not observed in cells without HER2 expression, suggesting that interference with HER2 could be used as an effective strategy for the treatment of breast cancer with high expression of HER2(Faltus *et al*, 2004).

### 3.7 Osteopontin

Osteopontin (OPN) is a secretory extracellular matrix protein and a cytokine as well. It can promote cell chemotaxis, adhesion and migration. More studies (Wai&Kou, 2008; Wise & King, 2008; Ramaiah&Rittling,2008) have demonstrated that OPN can interact with  $\alpha V\beta 3$  and CD44 to participate in cell inflammatory response, promote cell migration, adhesion and transduction of related signals, regulate the expression of genes related to tumor development and progression, promote degradation of extracellular matrix, suppress the immune function of the body against tumors, and accelerate tumor progression. Studies(Coppola *et al*, 2004; Cui *et al*, 2007;Tang *et al*, 2007)have found that the level of OPN expression is generally increased in lung, gastrointestinal, urogenital and gynecological tumors. Zhang (Zhang *et al*, 2010) used plasmid-mediated OPN-target siRNA to transfect lung cancer A549 cells and found that the invasion and proliferation abilities of A549 cell decreased. Gong (Gong *et al*, 2010) used PEI as the carrier of OPN-siRNA and injected it between tumor tissues in nude mice bearing transplanted gastric cancer tumors. It was found that the tumor size of the experiment group was only one-third of the control group, and survival of the mice was also prolonged (animals in the control group survived 40 days, and 50% of the animals in the experiment group remained surviving at 60 days).

### 3.8 $\beta$ -catenin

$\beta$ -catenin is a multi-functional adapter protein encoded by human CTNNB1 gene. Its function is expressed in two aspects: One is acting as an important component of the Wnt signaling pathway to mediate signal movement from the membrane to the cytoplasm and to the nucleus, regulating transcription of target genes (such as c-myc,cyclin D1, MMP-7 and cyclooxygenase-2). The other is participating in E-cadherin-mediated intercellular adhesion.  $\beta$ -catenin participates in cell growth, proliferation, invasion and metastasis in malignant tumor tissues. It was found that  $\beta$ -catenin expression was extraordinarily high in many tumors, especially in esophageal cancer. Wang (Wang *et al*, 2009,2010) used  $\beta$ -catenin-targeted RNA to interfere with plasmid pGen-3-CTNNB1 and found that pGen-3-CTNNB1 could lower the expression level of  $\beta$ -catenin in esophageal cancer tissue, and reduce the invasion and metastasis abilities of cancer cells. The result of the experiment with nude mice bearing transplanted esophageal cancer showed that the mean volume of tumors in  $\beta$ -catenin RNAi plasmid group was 909.3mm<sup>3</sup> versus 2684.4mm<sup>3</sup> in the negative control group and 2722.6mm<sup>3</sup> in the non-intervention group, indicating that  $\beta$ -catenin-target RNAi could inhibit the growth of esophageal cancer tumors.



### 3.9 MYC

Myc gene is a group of cancer genes discovered earlier, including C-myc, N-myc and L-myc, belonging to nucleoprotein-coding cancer gene. All the three genes encode a nuclear DNA-binding protein related to cell cycle regulation. The Myc gene family and its products can promote cell proliferation, immortalization, dedifferentiation and transformation, and play an important role in the formation of various tumors. Super-expression of Myc gene has been detected in gastric cancer, breast cancer, colon cancer, cervical cancer, Hodgkin's disease and head tumors. Zhang (Zhang *et al*, 2009) injected Myc-target siRNA between transplanted colon cancer tissues in nude mice, and found that the expression level of Myc decreased by 40%; large patches of necrosed cells were seen in tumor tissues; the proliferation of tumor cells was inhibited; and the mean tumor size was about 50% of the control group.

### 3.10 Epithelial cellular adhesion molecule

Epithelial cellular adhesion molecule (EpCAM) is a recognized tumor-related protein encoded by TACSTD1 gene. EpCAM is more than an adhesion molecule, and also has many other functions including participation in regulating cell migration, metastasis, differentiation, signal transduction, cell cycle and metabolism. Studies have demonstrated that EpCAM presents high expression in most solid tumors. In addition, the level of EpCAM expression is positively correlated with lymphatic metastasis. Du (Du *et al*, 2009) transfected lipofectamine 2000 with EpCAM-target siRNA to treat two gastric cell lines (AGS and SGC7901) in transplanted tumor-bearing nude mice, and found that the size of both tumors was about 50% smaller than that of the control group. They also found that the level of CCND1 expression in tumor tissues was decreased, and cell division was blocked at G1.

### 3.11 CD147

CD147 is a transmembrane glycoprotein extensively expressing in various tissues of the human body, belonging to the immunoglobulin super-family participating in various physiological and pathological processes of the body. CD147 interacts with integrin, central avidin, monocarboxylate transporter 1 (MCT1), MCT3 and MCT4 proteins. It is also related to the expression of lactate transporters. These proteins may act as candidate ligands or receptors of CD147 molecule and mediate the biological function of epithelial cells via protein-protein interactions. High expression of CD147 is detected in multiple tumor tissues, especially in pancreatic cancer (Zhang *et al*, 2007), where it promotes invasion, metastasis and anchorage-independent growth of tumor cells, and induces tumor angiogenesis. Schneiderhan (Schneiderhan *et al*, 2009) used CD147-target RNAi to treat pancreatic cancer cells and found that MCT1 and MCT4 were inhibited in tumor cells, and the intracellular concentration of lactate was increased, thus inhibiting the growth of tumor cells. In vivo experiments using shRNA-target silencing of CD147 to treat transplanted pancreatic cancer tumors in nude mice showed that the tumor size of the experiment group was significantly smaller than that of the control group (39.7mm<sup>3</sup> vs 89.7mm<sup>3</sup>), indicating that CD147 as a helper protein could also be used as the target for tumor treatment.

### 3.12 Urokinase-type plasminogen activator

Urokinase-type plasminogen activator (uPA) is an important component participating in degradation of extracellular matrix and plays an important role in invasion and metastasis of tumor cells. Super-expression of uPA receptor (uPAR or PLAUR) is detected in many malignant tumor cells. Zhou (Zhou *et al*, 2009) used a retroviral vector carrying siRNA expressing target PLAUR to treat transplanted oral squamous cell carcinoma for 30 days, and found that the mean tumor size of the intervention group was 1382mm<sup>3</sup> versus 4181mm<sup>3</sup> in the NS control group. The number of apoptotic cells in the intervention group was significantly greater than that in the control group (32.7 vs 2.7,  $P < 0.01$ ). The expression level of u-PAR, MMP2, MMP9, VEGFD, VEGFC and VEGFR-3 in the intervention group decreased significantly, and proliferation-related Ki-67 was inhibited.

### 3.13 P21-activated kinase 6

Super-expression of P21-activated kinase 6 (encoded by PAK6) is detected in malignant prostate cancer tissues. Wen (Wen *et al*, 2009) compared the inhibitory effect of PAK6-target siRNA (by intratumor injection in transplanted pancreatic cancer tumor-bearing nude mice) and Taxol on tumors, where siRNA was injected into tumors of transplanted pancreatic cancer-bearing nude mice. The result of six-week observation showed that the mean tumor size of the control group, Taxol group, siRNA group and Taxol+siRNA group was 475mm<sup>3</sup>, 210mm, 68mm<sup>3</sup> and 47mm<sup>3</sup> respectively. PAK6-siRNA inhibited the growth of pancreatic cancer cells and blocked them at G2-M, demonstrating that both Taxol and PAK6-siRNA could inhibit tumor growth. The inhibitory effect of combined use of Taxol and PAK6-siRNA was significantly better than either of the two along.

### 3.14 Skp-2

Skp-2 is a substrate recognition subunit of ubiquitin-protein ligase complex SCF/Skp-2, high expression of which in many tumors suggests a poor prognosis. Increased Skp-2 is believed to be associated with cell cycle disturbances in many tumors. HIV retroviral vector-mediated RNAi inhibited Skp-2 high expression cells effectively by increasing the expression of p27 and p21, but it had no significant effect on Skp-2 low expression cells (Sumimoto *et al*, 2005). Intervention of Skp-2 expression in 90% A549 and H1792 lung cancer cell strains could reduce p27 expression, induce apoptosis and inhibit proliferation (Simpson *et al*, 2004). Subcutaneous injection of the adenoviral vector of Skp-2 RNAi could inhibit the growth of subcutaneously transplanted lung cancer effectively, but it was ineffective in tumors without high expression of Skp-2 (Faltus *et al*, 2004). Sumimoto *et al* (Sumimoto *et al*, 2006) also found that interference with Skp-2 and BRAFD expression could inhibit the growth and invasion of melanoma cells in vitro.

### 3.15 Cytokine-induced apoptosis-inhibiting molecule

Cell apoptosis is programmed cell death regulated by multiple genes. Cytokine-induced apoptosis inhibitor 1 (CIAPIN1) is an important regulator of cell apoptosis. It has a significant inhibitory effect on multiple links in cell apoptotic pathways and a positive regulatory effect on Ras and other survival signaling pathways, thus promoting cell survival and proliferation (Shibayama *et al*, 2004). In recent years, many studies on the relationship

between CIAPIN1 and tumor formation showed that CIAPIN1 expression was increased significantly in HCC, gastric cancer and other malignant tumor tissues (Ida *et al*, 2007; Zhang *et al*, 2008). Li (Li *et al*, 2008) used adenovirus-mediated RNAi to knock out CIAPIN1 in HCC cells and found that CIAPIN1 gene expression in HCC cells was down-regulated, where cells were prevented from entering phase S and cell proliferation was inhibited. Eight weeks after injection of adenovirus/siRNA between transplanted tumor tissues, the tumor size was only one-sixth of the control group, and pathological sections showed apoptotic cells in tumor tissues.

### 3.16 Eukaryotic initiation factor 4E

Available studies showed that any form of genetic variation should function at the protein level (Branhart&Simon, 2007). Therefore the relationship between eukaryotic cell (protein translation) initiation factor and tumors has aroused increasing attention, especially eukaryotic initiation factor 4E (eIF4E), which was believed to be more closely related to tumors (Sonenberg, 2008). Data have shown that eIF4E can alter the amount of expression of some malignant tumor-related genes and is the determinant of malignant phenotype production. It promotes cells to surpass the limit of normal growth and undergo carcinogenesis through regulating the amount of translation of some specific malignancy-related molecules (Culjkovic *et al*, 2006). Dong (Dong *et al*, 2009) used a vector carrying shRNA of survivin-targeted eIF4E to transfect human breast cancer cells, and found that not only the level of eIF4E mRNA and protein expression was decreased but tumor progression-related protein VEGF, FGF2 and CCND1 were also down-regulated markedly. The breast cancer tumor size of the intervention group using eIF4E-targeted shRNA was significantly smaller than that of the control group (233.5mm<sup>3</sup> vs 397.7mm<sup>3</sup>,  $P < 0.01$ ). In addition, shRNA could also enhance the chemotherapy effect of cisplatin. The result showed that the tumor size of the cisplatin+shRNA plasmid combination group was significantly smaller than that of the plasmid control group (134.5mm<sup>3</sup> vs 208.9mm<sup>3</sup>,  $P < 0.01$ ).

### 3.17 Erythropoietin

Erythropoietin (EPO) was initially found to play a main regulatory role in the proliferation and differentiation of erythroid cells. Studies have shown that t EPO and erythropoietin receptor (EPOR) are expressed in many different nonhematopoietic organs and tissues, playing a role in promoting angiogenesis and protecting tissues. Many recent studies have discovered extensive expressions of EPO and EPOR in many malignant tumors, the autocrine/paracrine pathways of which are associated with tumor microangiogenesis, stimulation of tumor cell proliferation and sensitivity to chemotherapy. In addition, Paragh (Paragh *et al*, 2009) found that EPOR had a function independent of EPO in tumors. It was found that EPOR was highly expressed in ovarian cancer A2780 cell line, but no expression of EPO was observed in normal or hypoxic conditions. Use of exogenous EPO to intervene 2780 cells did not alter their biological effect. Paragh (Paragh *et al*, 2009) transplanted ovarian cancer cells treated with EPOR-target shRNA and those with a negative control vector in mice subcutaneously for 7 weeks, and found that the mean tumor size of the negative control group was 10-fold larger than that of the intervention group. This difference in tumor size is mainly due to decreased cell proliferation in the intervention group. However, the apoptotic rate was

similar between the two groups, indicating that EPOR plays a role in regulating cell proliferation, and this effect is independent of EPO.

### 3.18 miR-221 and miR-222

miR-221 and miR-222 are miRNA in cluster distribution with high homogeneity. Studies have shown that tumor suppressor gene p27 (Fornari *et al*, 2008), p53 (Fornari *et al*, 2008), pro-apoptotic factor Bim and Bmf (Gramantieri *et al*, 2009) are all target genes of miR-222/miR-221. studies in recent years have shown that miR-221 and miR-222 participate in the development of bladder cancer, HCC, prostate cancer and melanoma. Artificially synthesized small RNA precisely compensatory with miRNA can be used as an miRNA inhibitor to silence endogenous miRNA. Mercatelli *et al* (Mercatelli *et al*, 2008) used anti miR-221 and anti miR-222 inhibitors and a negative control inhibitor to intervene transplanted prostate cancer in nude mice for 33 days, and found that the mean tumor size of the intervention group and the control group was 197.2mm<sup>3</sup> and 276.82mm<sup>3</sup> respectively, indicating that regulation of miR221/222 via RNAi could be used as the target of tumor treatment.

### 3.19 miR-16

miR-16 can target Oncogene BCL-2. By regulating BCL-2 expression with miR-16, tumor cell proliferation can be inhibited. Schaefer *et al* (Schaefer *et al*, 2010) found that miR-16 can down-regulate BCL-2 expression in prostate cancer cells. Takeshita *et al* (Takeshita *et al*, 2010) used chemically synthesized miR-16 to transfect prostate cancer cell line 22Rv1, Du145, PPC-1 and PC-3M-luc, and found that the proliferation of these cells was inhibited. They administered miR-16 and atelocollagen to prevent bone metastasis from transplanted prostate cancer tumor in nude mice via tail vein injection. The results showed that, compared to i.v. injection of atelocollagen as a positive control, miR-16 halted the progression of the transplanted tumors and metastatic lesions, and that miR-16 could regulate the expression of CDK1 and CDK2 that participate in cell cycle and proliferation. Therefore, intravenous administration of miR-16 could be applied as a strategy for the treatment of advanced prostate cancer.

### 3.20 miR-26a

miR-26a is an miRNA that plays role of tumor suppressor gene. Lu (Lu *et al*, 2011) found that miR-26a could down-regulate the level of EZH2 expression in nasopharyngeal cancer cells, inhibit cell proliferation significantly, and block cell division at G1. Kota *et al* (Kota *et al*, 2009) used miR-26a to intervene HCC, and found that miR-26a could down-regulate the level of cyclin CCND2 and CCNE2 expression, inhibit the proliferation of HCC cells in vitro, and inhibit tumor progression in vivo, indicating that miR-26a could be used as a strategy for tumor treatment by down-regulating cancer genes.

### 3.21 Specific mutation ectopic gene

1. Ras: Ras is the gene that is most likely to undergo mutation in human malignant tumors. A single-point mutation is enough to transform normal protein into cancer protein. Therefore, there is mild difference in DNA sequence between the wild-type Ras

and mutated-type Ras. Previous methods of gene therapy were unable to inhibit the expression of mutated-type Ras effectively. Researchers have succeeded in using retrovirus-mediated RNA intervention technique to inhibit the expression of mutant K-rasV12 without affecting the other Ras subtypes (Brummelkamp *et al*, 2002). It not only inhibited neoplastic growth and transformation in experiments *in vitro* but obtained the same result in animal models.

2. bcr-abl: chronic myeloid leukemia (CML) is associated with chromosomal translocation. Protein tyrosine kinase encoded by fusion gene bcr-abl formed during translocation plays a very important role in the development of CML. Many studies on bcr-abl interference showed that the interference was effective on fusion gene, as shown by down-regulated expression of bcr-abl protein expression, inhibition on cell growth and apoptosis, increased sensitivity to the anti-cancer agent imatinib mesylate, while it had no effect on the wild-type gene in normal tissues. Koldehoff *et al* (Koldehoff *et al*, 2007) first reported the successful use of bcr-abl siRNA in the human body. It enhanced the sensitivity to the anti-cancer agent imatinib mesylate, inhibited the expression of bcr-abl, and promoted cell apoptosis. However, siRNA directing at bcr-abl homologous chromosomes could not enhance the sensitivity to imatinib mesylate and had no effect at all (Lima *et al*, 2004). The reason may be due to different degrees of down-regulation of bcr-Abl protein expression by the siRNA.
3. p53: 50% tumors have mutated-type p53, which attenuates the function of the wild-type. Inhibiting the expression of the mutated-type p53 can help recover the function of the wild-type. A large-scale study on loss of gene function by using RNA Databank (Berns *et al*, 2004) revealed one known and five new p53-dependent hyperplasia suppressor genes (HSG). Inhibition on the expression of these genes can counteract the anti-proliferation function of p53 and p19ARF, and thus remove G1 arrest arising from DNA injury.

#### 4. Clinical trials

Local and systemic drug administrations are the main routes of drug delivery in current clinical trials *in vivo*. Local drug administration depends on the type of target tissue, including intravitreal injection for ophthalmological diseases; intranasal administration for respiratory diseases caused by respiratory syncytial virus; intracranial injection for central nervous system diseases; intratumor injection for carcinogenic genes; and intramuscular injection for muscle tissue diseases such as rheumatoid arthritis. Drugs delivered by local administration can directly act on the target organ, thus reducing the probability of degradation of siRNA by nuclease and the dosage of siRNA. Systemic drug administration mainly depends on intraperitoneal and intravenous injection. Systemic drug administration often needs more siRNA to enable the target organ or tissue to obtain an efficacious drug concentration.

A lot of studies have demonstrated that RNAi has substantial potential for tumor treatment. Several RNAi agents have entered clinical trials in the world (Table 1). In May 2008, RNAi agent CALAA-01 for the treatment of solid tumors, developed by Calando Pharmaceuticals, was approved by the Food and Drug Administration (FDA) of the United States for Phase I clinical trial. CALAA-01 is a complex of siRNA and cyclodextrin-transferrin-adamant-PEG nanoparticles, in which human transferrin can specifically interact with transferrin receptor

that is overexpressed on the tumor cell membrane, thereby triggering the entry of the nanoparticles into tumor cells through receptor-mediated endocytosis. The target gene is ribonucleotide reductase subunit 2 (RRM2). After systemic administration by i.v. injection, siRNA efficiently inhibited tumor growth by down-regulating RRM2 expression (Davis,2009). It was also found that the levels of RRM2 mRNA and RRM2 protein were both decreased in the tumor. To detect the efficiency of RNAi, researchers found that there existed large amounts of RRM2 mRNA degradation products at tumor site containing the nanoparticles(Davis *et al*, 2010). This is the first clinical trial on RNAi medical treatment of malignant tumors.

SiRNA agent	Company/Institution	Target	Disease	Clinical Trials
CALAA-01	Calando Pharmaceuticals	RRM2	Solid tumor	Phase II
ALN-VSP02	Alnylam	KSP, VEGF	hepatocarcinoma	Phase II
Anti-tat/rev shRNA	Benitec&The city of Hope National Medical Center	Tat & Rev from HIV	AIDS-related lymphoma	Phase I
Atu027	Silence Therapeutics	PKN3	Pulmonary carcinoma	Phase I
BCR-ABL siRNA	University of Duisburg-Essen	Bcr-able	Chronic myeloid leukemia	Single patient
FANG	Gradlis, Inc.	Furin	Advanced cancer	Phase I
SV40/BCR-ABL	Hadassah Medical Organization	Bcr-abl	Chronic myeloid leukemia	Phase I
siRNA immunotherapy	Duke University Hospital	Proteasome	Metastatic melanoma	Phase I

Table 1. A summary of current siRNA agent for clinical trials

Alnylam Pharmaceuticals (Alnylam Pharmaceuticals,2011a,2011b) conducted Phase I clinical trial by intravenous administration of ALN-VSP02 for the treatment of HCC, where the siRNA therapeutic was enveloped in liposome. A total of 58 patients were recruited in the study and administered with the therapeutic at an interval of two weeks. The therapeutic agent targets kinesin spindle protein (KSP) and VEGF that are highly expressed in tumor cells. KSP plays a key role in cell mitosis and is the dynein necessary for bipolar spindle formation and maintenance. Inhibition of KSP would arrest the cell cycle. VEGF promotes angiogenesis, and therefore inhibition of VEGF would suppress tumor growth. Currently, Phase I clinical trial with ALN-VSP02 has been done, and Phase II clinical trial is to be started in 2012.

Recently, Silence Therapeutics has started Phase I clinical trial on Atu027(Aleku et al, 2008; Santel et al, 2010). Atu027 is a lipid-based siRNA complex containing target protein kinase N3 (PKN3), which participates in tumor occurrence and plays an important role in tumor metastasis. Atu027 can inhibit tumor progression by silencing the expression of PKN3. Pre-clinical experiments demonstrated that Atu027 could prevent breast cancer cells from

spreading to the lungs in mice. In addition, Atu027 could apparently inhibit the growth of transplanted prostate cancer in nude mice, where the tumor size was about 50% that of the control group; the number of metastatic lymph nodes was only 50% that of the control group; and the lymphatic density in the tumor area was also decreased markedly, though there was no significant difference in vascular density in the tumor area between the study and control groups. In a toxicity experiment, the researchers injected different doses (0.3, 1.0 and 3.0mg/kg) of Atu027 to different groups of cynomolgus monkeys at a 4-day interval, and no significant toxic and adverse effects were observed. Phase I clinical trial with Atu027 mainly showed safety, tolerance and pharmacokinetics.

## 5. Conclusion

The understanding of signaling pathways participating in the development and progression of malignant tumors and the development of specific target RNAi technique have offered hope to conduct individualized regimens for tumor treatment. However, current RNAi therapeutic approaches are immature and many problems need to be tackled, such as targeting and off-target effects, drug delivery systems, drug administrations and safety. As siRNA with a sequence of only 20-nucleotides could induce a tremendous effect of gene silencing, the sequence of siRNA must be selected accurately in the process of designing siRNA sequences to avoid the off-target effect. Additionally, a mRNA target sequence may hide in the mRNA secondary structure or folding region, making it impossible for siRNA to silence the mRNA. It is therefore necessary to conduct experiments repeatedly to select a suitable target site. Before RNAi medical treatment can be used clinically, it is necessary to seek an efficient delivery system and rational drug administration. In addition, as nucleotide sequences used for RNAi have no cell targetability and are likely to degrade, it is necessary to design and synthesize a drug delivery system that is non- or low toxic to normal cells and can deliver siRNA to tumor cells efficiently.

## 6. Acknowledgment

This work was financially supported by the Key Scientific Project of Shanghai Putuo District(PTKW10-B01), the Young Program of Shanghai health bureau(2009Y127), Young backbone promotion plan of Shanghai society of Nuclear Medicine (2009-NM-07).

## 7. References

- Aleku, M.; Schulz, P.; Keil, O.; Santel, A.; Schaeper, U.; Dieckhoff, B.; Janke, O.; Endruschat, J.; Durieux, B.; Roder, N.; Löffler, K.; Lange, C.; Fechtner, M.; Mopert, K.; Fisch, G.; Dames, S.; Arnold, W.; Jochims, K.; Giese, K.; Wiedenmann, B.; Scholz, A.; Kaufmann, J.(2008). Atu027, a liposomal small interfering RNA formulation targeting protein kinase N3, inhibits cancer progression, *Cancer Research.*, Vol.68, No.23, (December 2008), pp.9788-9798, ISSN0008-5472.
- Alnylam Pharmaceuticals. (August 2011).Dose Escalation trial to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of intravenous ALN-VSP02

- in patients with advanced solid tumors with liver involvement, 23.08.2011, Available from <http://clinicaltrials.gov/show/NCT00882180>.
- Alnylam Pharmaceuticals. (June 2011).Alnylam Presents Phase I Data For ALN-VSP, An RNAi Therapeutic For The Treatment Of Liver Cancers, At ASCO Meeting, 06.06.2011, Available from <http://www.medicalnewstoday.com/releases/227569.php>
- Berns, K.; Hijmans, E.; Mullenders, J.; Brummelkamp, T.; Velds, A.; Heimerikx, M.; Kerkhoven, R.; Madiredjo, M.; Nijkamp, W.; Weigelt, B.; Agami, R.; Ge, W.; Cavet, G.; Linsley, P.; Beijersbergen, R.; Bernards, R.(2004). A large-scale RNAi screen in human cells identifies new components of the p53 pathway, *Nature*, Vol.428, No.6981, (March 2004), pp.431-437, ISSN 0028-0836
- Berns, K.; Hijmans, E.; Mullenders, J.; Brummelkamp, T.; Velds, A.; Heimerikx, M.; Kerkhoven, R.; Madiredjo, M.; Nijkamp, W.; Weigelt, B.; Aqami, R.; Ge, W.; Cavet, G.; Linsley, P.; Beijersbergen, R.; Bernards, R.(2004). A large-scale RNAi screen in human cells identifies new components of the p53 pathway, *Nature*, Vol.428, No.6981, (March 2004), pp.431-437, ISSN 0028-0836.
- Bernstein, E.; Caudy, A.; Hammond, S.; Hannon, G.(2001). Role for a bidentate ribonuclease in the initiation step of RNA interference, *Nature*, Vol.409, No. 6818, (January 2001), pp.363-366, ISSN 0028-0836
- Blaszczyk, J.; Tropea, J.; Bubunenko, M.; Routzahn, K.; Waugh, D.; Court, D.; Ji, X. (2001). Crystallographic and modeling studies of RNase III suggest a mechanism for double-stranded RNA cleavage. *Structure*, Vol.79, No. 12, (December 2001), pp.1225-1236, ISSN 0969-2126
- Branhart, B.; Simon, M.(2007). Taking aim at translation for tumor therapy, *The Journal of clinical investigation* , Vol.117, No.9, (September 2007), pp.2385-2388, ISSN 0021-9738.
- Brummelkamp, T.; Bernards , R.; Agami , R.(2002). Stable suppression of tumorigenicity by virus-mediated RNA interference, *Cancer Cell*, Vol.2, No.3, (September 2002), pp.243-247, ISSN 0007-9235.
- Brummelkamp, T.; Nijman, S.; Dirac, A.; Bernards, R. (2003).Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB, *Nature*, Vol.424, No.6950, (August 2003), pp.797-801, ISSN 0028-0836
- Chen, T.; Deng, C.(2008). Inhibitory effect of siRNA targeting survivin in gastric cancer MGC-803 cells, *International immunopharmacology*, Vol.8, No.7, (July 2008), pp.1006-1011, ISSN 1567-5769
- Collins, R.; Cheng, X. (2005).Structural domains in RNAi, *FEBS Lett*, Vol.579, No. 26, (October 2005), pp.5841-5849, ISSN 0014-5793
- Coppola, D.; Szabo, M.; Boulware, D.; Muraca, P.; Alsarraj, M.; Chambers, A.; Yeatman, T.(2004). Correlation of osteopontin protein expression and pathological stage across a wide variety of tumor histologies, *Clinical cancer research*, Vol.10, No.1(Pt1), (January 2004), pp.184-190, ISSN 1078-0432
- Cordin, O.; Banroques, J.; Tanner, N.; Linder, P.(2006) The DEAD-box protein family of RNA helicases. *Gene*, Vol.367, (February 2006), pp.17-37, ISSN 0378-1119.



- Cui, R.; Takahashi, F.; Ohashi, R.; Gu, T.; Yoshioka, M.; Nishio, K.; Ohe, Y.; Tominaga, S.; Takagi, Y.; Sasaki, S.; Fukuchi, Y.; Takahashi, K.(2007). Abrogation of the interaction between osteopontin and  $\alpha$ v $\beta$ 3 integrin reduces tumor growth of human lung cancer cells in mice, *Lung cancer*, Vol.57, No.3, (September 2007), pp.302-310, ISSN 0169-5002
- Culjkovic, B.; Topisirovic, I.; Skrabanek, L.; Ruiz-Gutierrez, M.; Borden, K.(2006). eIF4E is a central node of an RNA regulon that governs cellular proliferation, *The Journal of cell biology*, Vol.175, No.3, (November 2006), pp.415-426, ISSN 0021-9525.
- Davis, M.(2009). The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic, *Molecular Pharmaceutics*, Vol.6, No.3, (May-June 2009), pp.659-668, ISSN 1543-8384.
- Davis, M.; Zuckerman, J.; Choi, C.; Seligson, D.; Tolcher, A.; Alabi, C.; Yen, Y.; Heidel, J.; Ribas, A. (2010).Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles, *Nature*, Vol.464, No. 7291, (April 2010), pp. 1067-1070, ISSN 0028-0836
- Davis, M.; Zuckerman, J.; Choi, C.; Seligson, D.; Tolcher, A.; Alabi, C.; Yen, Y.; Heidel, J.; Ribas, A.(2010). Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles, *Nature*, Vol.464, No.7291, (April 2010), pp.1067-1070, ISSN 0028-0836.
- de Souza Nascimento, P.; Alves, G.; Fiedler, W.(2006). Telomerase inhibition by an siRNA directed against hTERT leads to telomere attrition in HT29 cells, *Oncology reports*, Vol.16, No.2, (August 2006), pp.423-428, ISSN 1021-335X
- Devi, G.(2006). siRNA-based approaches in cancer therapy, *Cancer gene therapy*, Vol.13, No.9, (September 2006), pp.819-829, ISSN 0929-1903
- Dong, K.; Wang, R.; Wang, X.; Lin, F.; Shen, J.; Gao, P.; Zhang, H.(2009).Tumor-specific RNAi targeting eIF4E suppresses tumor growth, induces apoptosis and enhances cisplatin cytotoxicity in human breast carcinoma cells, *Breast cancer research and treatment*, Vol.113, No.3, (February 2009), pp.443-456, ISSN 0167-6806.
- Du, W.; Wang, L.; Cao, S.; Chen, B.; Zhang, Y.; Bai, F.; Liu, J.; Fan, D.(2009). EpCAM is overexpressed in gastric cancer and its downregulation suppresses proliferation of gastric cancer, *Journal of cancer research and clinical oncology*, Vol.135, No.9, (September 2009), pp.1277-1285, ISSN 0171-5216
- Faltus, T.; Yuan, J.; Zimmer, B.; Krämer, A.; Loibl, S.; Kaufmann, M.; Strebhardt, K.(2004). Silencing of the HER2/neu gene by siRNA inhibits proliferation and induces apoptosis in HER2/neu-overexpressing breast cancer cells, *Neoplasia*, Vol.6, No.6, (November-December 2004), pp.786-795, ISSN 1522-8002
- Fire, A.; Xu, S.; Montgomery, M.; Kostas, S.; Dreiver, S.; Mello, C.(1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*, *Nature*, Vol.391, No.6669, (February 1998), pp. 806-811, ISSN 0028-0836.
- Fornari, F.; Gramantieri, L.; Ferracin, M.; Veronese, A.; Sabbioni, S.; Calin, G.; Grazi, G.; Giovannini, C.; Croce, C.; Bolondi, L.; Negrini, M.(2008). MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma, *Oncogene*, Vol.27, No.43, (September 2008), pp.5651-5661, ISSN 0950-9232.

- Gandellini, P.; Folini, M.; Bandiera, R.; De Cesare, M.; Binda, M.; Veronese, S.; Daidone, M.; Zunino, F.; Zaffaroni, N.(2007). Down-regulation of human telomerase reverse transcriptase through specific activation of RNAi pathway quickly results in cancer cell growth impairment, *Biochemical pharmacology*, Vol.73, No.11, (June 2007), pp.1703-1714, ISSN 0006-2952
- Gong, M.; Lu, Z.; Fang, G.; Bi, J.; Xue, X.(2008). A small interfering RNA targeting osteopontin as gastric cancer therapeutics, *Cancer letters*, Vol.272, No.1, (December 2008), pp.148-159, ISSN 0304-3835
- Gramantieri, L.; Fornari, F.; Ferracin, M.; Veronese, A.; Sabbioni, S.; Calin, G.; Grazi, G.; Croce, C.; Bolondi, L.; Neqrini, M. (2009).MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality, *Clinical cancer research*, Vol.15, No.16, (August 2009), pp.5073-5081, ISSN 1078-0432.
- Gregory, R.; Chendrimada, T.; Cooch, N.; Shiekhattar, R.(2005). Human RISC couples microRNA biogenesis and posttranscriptional gene silencing, *Cell*, Vol.123, No.4, (November 2005), pp.632-640, ISSN 0092-8674
- Guo, S.; Kemphues, K. (1995).par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed, *Cell*, Vol.81, No. 4, (May 1995), pp.611-620, ISSN 0092-8674
- Hallegger, M.; Taschner, A.; Jantsch, M. (2006).RNA aptamers binding the double-stranded RNA-binding domain.RNA, Vol.12, No. 11, (November 2006), pp.1993-2004, ISSN 1355-8382
- Hammond, S.; Bernstein, E.; Beach, D.; Hannon, G.(2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells, *Nature*, Vol.404, No.6775, (March 2000), pp.293-296, ISSN 0028-0836
- Hammond, SM. (2005).Dicing and slicing: the core machinery of the RNA interference pathway, *FEBS Letters*, Vol.579, No.26, (October 2005), pp.5822-5829, ISSN 0014-5793
- Hannon, G. (2002).RNA interference, *Nature*, Vol.418, No.6894, (July 2002), pp. 244-251, ISSN 0028-0836.
- He, X.; Liu, T.; Xiao, Y.; Feng, Y.; Cheng, D.; Ting, G.; Zhang, L.; Zhang, Y.; Chen, Y.; Ting, G.; Zhang, L.(2009). Vascular endothelial growth factor-C siRNA delivered via calcium carbonate nanoparticle effectively inhibits lymphangiogenesis and growth of colorectal cancer in vivo, *Cancer biotherapy & radiopharmaceuticals*, Vol.24, No.2, (April 2009), pp.249-259, ISSN 1084-9785
- Huang, C.; Jiang, T.; Zhu, L.; Liu, J.; Cao, J.; Huang, K.; Qiu, Z.(2011). STAT3-targeting RNA interference inhibits pancreatic cancer angiogenesis in vitro and in vivo, *International journal of oncology*, Vol.38, No.6, (June 2011), pp.1637-1644, ISSN 1019-6439
- Ida, H.; Yoshida, H.; Nakamura, K.; Yamaguchi, M.(2007). Identification of the *Drosophila* eIF4A gene as a target of the DREF transcription factor, *Experimental cell research*, Vol.313, No.20, (December 2007), pp.4208-4220, ISSN 0014-4827
- Jemal, A.; Bray, F.; Center, M.; Ferlay, J.; Ward, E.; Forman, D. (2011).Global cancer statistics, *Cancer Journal for Clinicians*, Vol.6, No.4, (Mar-Apr 2011), pp. 69-90, ISSN 0007-9235.

- Kanwar, J.; Kamalapuram, S.; Kanwar, R.(2010). Targeting survivin in cancer: patent review, *Expert opinion on therapeutic patents*, Vol.20, No.12, (December 2010), pp.1723-1737, ISSN 1354-3776
- Koldehoff , M.; Steckel , N.; Beelen , D.; Elmaagacli, A.; (2007). Therapeutic application of small interfering RNA directed against bcr-abl transcripts to a patient with imatinib-resistant chronic myeloid leukaemia, *Clinical and Experimental Medicine*, Vol.7, No.2, (June 2007), pp.47-55, ISSN 1591-8890.
- Koldehoff, M.; Elmaagacli, A. (2009).Therapeutic targeting of gene expression by siRNAs directed against BCR-ABL transcripts in a patient with imatinib-resistant chronic myeloid leukemia, *Methods in molecular biology*, Vol.487, (2009), pp. 451-466, ISSN 1064-3745
- Kota, J.; Chivukula, R.; O'Donnell, K.; Wentzel, E.; Montgomery, C.; Hwang, H.; Chang, T.; Vivekanandan, P.; Torbenson, M.; Clark, K.; Mendell, J.; Mendell, J.(2009).Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model, *Cell*, Vol.137, No.6, (June 2009), pp.1005-1017, ISSN 0092-8674.
- Krek, A.; Grün, D.; Poy, M.; Wolf, R.; Rosenberg, L.; Epstein, E.; MacMenamin, P.; da Piedade, I.; Gunsalus, K.; Stoffel, M.; Rajewsky, N. (2005).Combinatorial microRNA target predictions, *Nature genetics*, Vol.37, No.5, (May 2005), pp.495-500, ISSN 1061-4036
- Li, J.; Piao, Y.; Jiang, Z.; Chen, L.; , Sun, H. (2009) . Silencing of signal transducer and activator of transcription 3 expression by RNA interference suppresses growth of human hepatocellular carcinoma in tumor-bearing nude mice, *World journal of gastroenterology*, Vol.15, No.21, (June 2009), pp.2602-2608, ISSN 1007-9327
- Li, X.; Pan, Y.; Fan, R.; Jin, H.; Han, S.; Liu, J.; Wu, K.; Fan, D.(2008). Adenovirus-delivered CIAPIN1 small interfering RNA inhibits HCC growth in vitro and in vivo, *Carcinogenesis*, Vol.29, No.8, (August 2008), pp.1587-1593, ISSN 0143-3334
- Lima, R.; Martins, L.; Guimaraes, J.; Sambade, C.; Vasconcelos, M. (2004). Specific downregulation of bcl-2 and xIAP by RNAi enhances the effects of chemotherapeutic agents in MCF-7 human breast cancer cells, *Cancer Gene Therapy*, Vol.11, No.5, (March 2004), pp.309-316, ISSN0929-1903.
- López-Fraga, M.; Martínez, T.; Jiménez, A. (2009).RNA interference technologies and therapeutics: from basic research to products. *BioDrugs*, Vol.23, No.5, (October 2009), pp. 305-332, ISSN 1173-8804.
- Lu, J.; He, M.; Wang , L.; Chen, Y.; Liu, X.; Dong, Q.; Chen, Y.; Peng, Y.; Yao, K.; Kunq, H.; LI, X.; (2011). MiR-26a Inhibits Cell Growth and Tumorigenesis of Nasopharyngeal Carcinoma through Repression of EZH2, *Cancer research.*, Vol.71, No.1, (January 2011), pp.225-233, ISSN 0008-5472.
- Ma, J.; Ye, K.; Patel, D. (2004).Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain. *Nature*, Vol.429, No. 6989, (May 2004), pp.318-322, ISSN 0028-0836
- Ma, J.; Yuan, Y.; Meister, G.; Pei, Y.; Tuschl, T.; Patel, D.(2005). Structural basis for 5'-end-specific recognition of guide RNA by the A. fulgidus Piwi protein, *Nature*, Vol.434, No.7033, (March 2005), pp.666-670, ISSN 0028-0836

- Macrae, I.; Li, F.; Zhou, K.; Cande, W.; Doudna, J.(2006). Structure of Dicer and echanistic implications for RNAi, *Cold Spring Harbor symposia on quantitative biology*, Vol.71, (2006), pp.73-80, ISSN 0091-7451
- Maples, P.; Kumar, P.; Yu, Y.; Wang, Z.(2009).FANG vaccine: autologous tumor vaccine genetically modified to express GMCSF and block production of furin, *BioProcessing Journal*, Vol.8, (2009), pp. 4-14, ISSN 1538-8786
- Matranga, C.; Tomari, Y.; Shin, C.; Bartel, D.; Zamore, P. (2005).Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes, *Cell*, Vol.123, No.4, (November 2005), pp.607-620, ISSN 0092-8674
- Meister G, Landthaler M, Peters L, Chen PY, Urlaub H, Lührmann R, Tuschl T.(2005). Identification of novel argonaute -associated proteins, *Current biology*, Vol.15, No.23, (December 2005), pp.2149-2155, ISSN 0960-9822
- Meister, G.; Landthaler, M.; Patkaniowska, A.; Dorsett, Y.; Teng, G.; Tuschl, T.(2004). Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs, *Molecular cell*, Vol.15, No.2, (July 2004), pp.185-197, ISSN 1097-2765
- Meister, G.; Tuschl, T. (2004).Mechanisms of gene silencing by double-stranded RNA, *Nature*, Vol.431, No.7006, (September 2004), pp.343-339, ISSN 0028-0836
- Mercatelli, N.; Coppola, V.; Bonci, D.; Miele, F.; Costantini, A.; Guadagnoli, M.; Bonanno, E.; Muto, G.; Frajese, G.; De, R.; Spagnoli, L.; Farace, M.; Ciafre, S.(2008). The inhibition of the highly expressed miR-221 and miR-222 impairs the growth of prostate carcinoma xenografts in mice, *PLoS One*, Vol.3, No.12, (December 2008), pp.4029, ISSN 1932-6203.
- Paccione, R.; Miyazaki, H.; Patel, V.; Waseem, A.; Gutkind, J.; Zehner, Z.; Yeudall, W.(2008). Keratin down-regulation in vimentin-positive cancer cells is reversible by vimentin RNA interference, which inhibits growth and motility, *Molecular cancer therapeutics*, Vol.7, No.9, (September 2008), pp.2894-2903, ISSN 1535-7163
- Paddison, P.; Silva, J.; Conklin, D.; Schlabach, M.; Li, M.; Aruleba, S.; Balija, V.; O'Shaughnessy, A.; Gnoj, L.; Scobie, K.; Chang, K.; Westbrook, T.; Cleary, M.; Sachidanandam, R.; McCombie, W.; Elledge, S.; Hannon, G.(2004). A resource for large-scale RNA-interference-based screens in mammals, *Nature*, Vol.428, No.6981, (March 2004), pp.427-431, ISSN 0028-0836
- Paragh , G.; Kumar, S.; Rakosy, Z.; Choi, S.; Xu, X.; Acs, G.(2009). RNA interference-mediated inhibition of erythropoietin receptor expression suppresses tumor growth and invasiveness in A2780 human ovarian carcinoma cells, *The American journal of pathology*, Vol.174, No.4, (April 2009), pp.1504-1514, ISSN 0002-9440.
- Pillai, R.; Bhattacharyya, S.; Filipowicz, W.(2007). Repression of protein synthesis by miRNAs: how many mechanisms? *Trends in cell biology*, Vol.17, No.3, (March 2007), pp.118-126, ISSN 0962-8924
- Preall, JB.; Sontheimer, EJ.(2005). RNAi: RISC gets loaded, *Cell*, Vol.123, No.4, (November 2005), pp.543-545, ISSN 0092-8674
- Ramaiah, S.; Rittling, S.(2008). Pathophysiological role of osteopontin in hepatic inflammation, toxicity, and cancer, *Toxicological sciences*, Vol.103, No.1, (May 2007), pp.4-13, ISSN 1096-6080

- Rao, D.; Maples, P.; Senzer, N.; Kumar, P.; Wang, Z.; Pappen, B.; Yu, Y.; Haddock, C.; Jay, C.; Phadke, A.; Chen, S.; Kuhn, J.; Dylewski, D.; Scott, S.; Monsma, D.; Webb, C.; Tong, A.; Shanahan, D.; Nemunaitis, J.(2010). Enhanced target gene knockdown by a bifunctional shRNA: a novel approach of RNA interference, *Cancer gene therapy*, Vol.17, No.11, (November 2010), pp.780-791, ISSN 0929-1903
- Rao, D.; Vorhies, J.; Senzer, N.; Nemunaitis, J.(2009). siRNA vs. shRNA: similarities and differences, *Advanced drug delivery reviews*, Vol.61, No.9, (July 2009), pp.746-759, ISSN 0169-409X
- Robb, G.; Rana, T. (2007).RNA helicase A interacts with RISC in human cells and functions in RISC loading, *Molecular cell*, Vol.26, No.4, (May 2007), pp.523-537, ISSN 1097-2765
- Ryter, J.; Schultz, S. (1998).Molecular basis of double-stranded RNA-protein interactions: structure of a dsRNA-binding domain complexed with dsRNA, *The EMBO journal*, Vol.17, No.24, (December 1998), pp.7505-7513, ISSN 0261-4189
- Santel, A.; Aleku, M.; Röder, N.; Mopert, K.; Durieux, B.; Janke, O.; Keil, O.; Endruschat, J.; Dames, S.; Lange, C.; Eisermann, M.; Löffler, K.; Fechtner, M.; Fisch, G.; Vank, C.; Schaeper, U.; Giese, K.; Kaufmann, J. (2010). Atu027 prevents pulmonary metastasis in experimental and spontaneous mouse metastasis models, *Clinical Cancer Research*, Vol.16, No.22, (November 2010), pp.5469-5480, ISSN 1078-0432.
- Schaefer, A.; Jung, M.; Mollenkopf, H.; Waqner, I.; Stephan, C.; Jentzmik, F.; Miller, K.; Lein, M.; Kristiansen, G.; Junq, K.(2010), Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma, *International journal of cancer*, Vol.126, No.5, (March 2010), pp.1167-1176, ISSN 0020-7136.
- Schneiderhan, W.; Scheler, M.; Holzmann, K.; Marx, M.; Gschwend, J.; Bucholz, M.; Gress, T.; Seufferlein, T.; Adler, G.; Oswald, F.(2009). CD147 silencing inhibits lactate transport and reduces malignant potential of pancreatic cancer cells in in vivo and in vitro models, *Gut*, Vol.58, No.10, (October 2009), pp.1391-1398, ISSN 0017-5749
- Schwarz, D.; Tomari, Y.; Zamore, P. (2004).The RNA-induced silencing complex is a Mg<sup>2+</sup>-dependent endonuclease, *Current biology*, Vol.14, No.9, (May 2004), pp.787-791, ISSN 0960-9822
- Seth, S.; Matsui, Y.; Fosnaugh, K.; Liu, Y.; Vaish, N.; Adami, R.; Harvie, P.; Johns, R.; Severson, G.; Brown, T.; Takagi, A.; Bell, S.; Chen, Y.; Chen, F.; Zhu, T.; Fam, R.; Maciagiewicz, I.; Kwang, E.; McCutcheon, M.; Farber, K.; Charmley, P.; Houston, M.; So, A.; Templin, M.; Polisky, B. (2011).RNAi-based therapeutics targeting survivin and PLK1 for treatment of bladder cancer, *Molecular therapy* , Vol.19, No.5, (May 2011), pp.928-935, ISSN 1525-0016
- Sethupathy, P.; Corda, B.; Hatzigeorgiou, AG. (2006).TarBase: A comprehensive database of experimentally supported animal microRNA targets, *RNA*, Vol.12, No.2, (February 2006), pp.192-197, ISSN 1355-8382
- Shibayama, H.; Takai, E.; Matsumura, I.; Kouno, M.; Morii, E.; Kitamura, Y.; Takeda, J.; Kanakura, Y.(2004). Identification of a cytokine-induced antiapoptotic molecule anamorsin essential for definitive hematopoiesis, *The Journal of experimental medicine*, Vol.199, No.4, (February 2004), pp.581-592, ISSN 0022-1007

- Silva, J.; Li, M.; Chang, K.; Ge, W.; Golding, M.; Rickles, R.; Siolas, D.; Hu, G.; Paddison, P.; Schlabach, M.; Sheth, N.; Bradshaw, J.; Burchard, J.; Kulkarni, A.; Cavet, G.; Sachidanandam, R.; McCombie, W.; Cleary, M.; Elledge, S.; Hannon, G.(2005). Second-generation shRNA libraries covering the mouse and human genomes, *Nature genetics*, Vol.37, No.11, (November 2005), pp.1281-1288, ISSN 1061-4036
- Simpson, K.; Dugan, A.; Mercurio, A.(2004). Functional analysis of the contribution of RhoA and RhoC GTPases to invasive breast carcinoma, *Cancer research*, Vol.64, No.23, (December 2004), pp.8694-8701, ISSN 0008-5472
- Sonenberg, N.(2008). eIF4E, the mRNA cap-binding protein: from basic discovery to translational research, *Biochemistry and cell biology*, Vol.86, No.2, (April 2008), pp.178-183, ISSN 0829-8211
- Song, J.; Smith, S.; Hannon, G.; Joshua-Tor, L. (2004).Crystal structure of Argonaute and its implications for RISC slicer activity, *Science*, Vol.305, No.5689, (September 2004), pp.1434-1437, ISSN 0036-8075
- Sumimoto, H.; Hirata, K.; Yamagata, S.; Miyoshi, H.; Miyagishi, M.; Taira, K.; , Kawakami, Y.(2006). Effective inhibition of cell growth and invasion of melanoma by combined suppression of BRAF (V599E) and Skp2 with lentiviral RNAi, *International journal of cancer*, Vol.118, No.2, (January 2006), pp.472-476, ISSN 0020-7136
- Sumimoto, H.; Yamagata, S.; Shimizu, A.; Miyoshi, H.; Mizuguchi, H.; Hayakawa, T.; Miyagishi, M.; Taira, K.; Kawakami, Y.(2005). Gene therapy for human small-cell lung carcinoma by inactivation of Skp-2 with virally mediated RNA interference, *Gene therapy*, Vol.12, No.1, (January 2005), pp.95-100, ISSN 0969-7128
- Takeshita, F.; Patrawala, L.; Osaki, M.; Takahashi, R.; Yamamoto, Y.; Kosaka, N.; Kawamata, M.; Kelnar, K.; Bader, A.; Brown, D.; Ochiya, T. (2010). Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes, *Molecular Therapy*, Vol.18, No.1, (January 2010), pp.181-187, ISSN 1525-0016.
- Tang, G. (2005).siRNA and miRNA: an insight into RISCs, *Trends in biochemical sciences*, Vol.30, No.2, (February 2005), pp.106-114, ISSN 0968-0004
- Tang, H.; Wang, J.; Bai, F.; Hong, L.; Liang, J.; Gao, J.; Zhai, H.; Lan, M.; Zhang, F.; Wu, K.; Fan, D.(2007). Inhibition of osteopontin would suppress angiogenesis in gastric cancer, *Biochemistry and cell biology*, Vol.85, No.1, (February 2007), pp.103-110, ISSN 0829-8211
- Tomari, Y.; Matranga, C.; Haley, B.; Martinez, N.; Zamore, P.(2004). A protein sensor for siRNA asymmetry, *Science*, Vol.306, No.5700, (November 2004), pp.1377-1380, ISSN 0036-8075
- Wai PY, Kuo PC.(2008). Osteopontin: regulation in tumor metastasis, *Cancer metastasis reviews*, Vol.27, No.1, (March 2008), pp.103-108, ISSN 0167-7659
- Wang, J.; Ji, A.; Wen, J.; Ren H.(2010). Effect of  $\beta$ -catenin gene silencing by shRNA on biologic characteristics of human esophageal carcinoma cells, *Chinese Journal of Pathology*, Vol.39, No.12, (December 2010), pp.835-841, ISSN 0529-5807
- Wang, J.; Zheng, C.; Wang, Y.; Wen, J.; Ren, H.; Liu, Y.; Jiang, H.(2008).Gene silencing of beta-catenin by RNAi inhibits cell proliferation in human esophageal cancer cells in

- vitro and in nude mice, *Diseases of the esophagus*, Vol.22, No.2, (April 2009), pp.151-162, ISSN 1120-8694
- Wang, Q.; Lv, Y.; Fei, L.(2010). New Thoughts on Cancer Gene Therapy, *Chinese Journal of Clinical Oncology*, Vol.37, No.15, (August 2010), pp. 893-896, ISSN 1000-8179.
- Wen, X.; Li, X.; Liao, B.; Liu, Y.; Wu, J.; Yuan, X.; Ouyang, B.; Sun, Q.; Gao, X.(2009). Knockdown of p21-activated kinase 6 inhibits prostate cancer growth and enhances chemosensitivity to docetaxel, *Urology*, Vol.73, No.6, (June 2009), pp.1407-1411, ISSN 0090-4295
- Wise, G.; King, G.(2008). Mechanisms of tooth eruption and orthodontic tooth movement, *Journal of dental research*, Vol.87, No.5, (May 2008), pp.414-434, ISSN 0022-0345
- Wu, H.; Henras, A.; Chanfreau, G.; Feigon, J. (2004).Structural basis for recognition of the AGNN tetraloop RNA fold by the double-stranded RNA-binding domain of Rnt1p RNase III. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.101, No.22, (June 2004), pp.8307-8312, ISSN 0027-8424
- Xv, N.; Dong, Z.(2003). Overview of the Cancer Epidemiological Status in China and Strategy on Cancer Control, *China Journal of Cancer Prevention and treatment*, Vol.10, No.1, (January 2003), pp. 71-74, ISSN 1673-5269.
- Yuan, Y.; Pei, Y.; Ma, J.; Kuryavyyi, V.; Zhadina, M.; Meister, G.; Chen, H.; Dauter, Z.; , Tuschl, T.; Patel, D. (2005).Crystal structure of A. aeolicus argonaute, a site-specific DNA-guided endoribonuclease, provides insights into RISC-mediated mRNA cleavage, *Molecular cell*, Vol.19, No.3, (August 2005), pp.405-419, ISSN 1097-2765
- Zeng, P.; Wagoner, H.; Pescovitz, O.; Steinmetz, R.(2005). RNA interference (RNAi) for extracellular signal-regulated kinase 1 (ERK1) alone is sufficient to suppress cell viability in ovarian cancer cells, *Cancer biology & therapy*, Vol.4, No.9, (September 2005), pp.961-967, ISSN 1538-4047
- Zhang, W.; Erkan, M.; Abiatari, I.; Giese, N.; Felix, K.; Kayed, H.; Büchler, M.; Friess, H.; Kleeff, J. (2007). Expression of extracellular matrix metalloproteinase inducer (EMMPRIN/CD147) in pancreatic neoplasm and pancreatic stellate cells, *Cancer biology & therapy*, Vol.6, No.2, (February 2007), pp.218-227, ISSN 1538-4047
- Zhang, X.; Ge, Y.; Tian, R.(2009). The knockdown of c-myc expression by RNAi inhibits cell proliferation in human colon cancer HT-29 cells in vitro and in vivo, *Cellular & molecular biology letters*, Vol.14, No.2, (January 2009), pp.305-318, ISSN 1425-8153
- Zhang, X.; Ladd, A.; Dragoescu, E.; Budd, W.; Ware, J.; Zehner, Z.(2009). MicroRNA-17-3p is a prostate tumor suppressor in vitro and in vivo, and is decreased in high grade prostate tumors analyzed by laser capture microdissection, *Clinical & experimental metastasis*, Vol.26, No.8, (September 2009), pp.965-979, ISSN 0262-0898
- Zhang, Y.; Lyver, E.; Nakamaru-Ogiso, E.; Yoon, H.; Amutha, B.; , Lee, D.; Bi, E.; Ohnishi, T.; Daldal, F.; Pain, D.; Dancis, A.(2008). Dre2, a conserved eukaryotic Fe/S cluster protein, functions in cytosolic Fe/S protein biogenesis, *Molecular and cellular biology*, Vol.28, No.18, (September 2008), pp.5569-5582, ISSN 0270-7306

- Zhang, B.; Zhang, Z.; Wang, C.(2010). Inhibitory effects on proliferation and invasion of lung cancer cells by RNAi-mediated knockdown of osteopontin, *Chinese Journal of experimental surgery*, Vol.27, No.2, (February 2010), pp.230-231, ISSN 1001-9030
- Zhang, S.; Chen, W.; Lei Z.; Zou, X.; Zhao, P. (2008).A Report of Cancer Incidence from 37 Cancer Registries in China, 2004, *Bulletin of Chinese Cancer*, Vol.17, No.11, (November 2011), pp. 909-912, ISSN 1004-0242.
- Zhou, H.; Tang, Y.; Liang, X.; Yang, X.; Yang, J.; Zhu, G.; Zheng, M.; Zhang, C.(2009). RNAi targeting urokinase-type plasminogen activator receptor inhibits metastasis and progression of oral squamous cell carcinoma in vivo, *International journal of cancer*, Vol.125, No.2, (July 2009), pp.453-462, ISSN 0020-7136





## **Biomedicine**

Edited by Dr. Chao Lin

ISBN 978-953-51-0352-3

Hard cover, 200 pages

**Publisher** InTech

**Published online** 21, March, 2012

**Published in print edition** March, 2012

Comprehensive and systematic scientific researches in cell biology and molecular biology have promoted the evolution of traditional medicine. Scientists are now able to interpret a few perplexed medical difficulties with the emphasis on molecular levels. This book focuses on frontier progress in modern biomedical science dealing with regenerative medicine, gene medicine, nanobiomedicine, and medical devices. Each chapter is intently chosen and written by professional experts in the field of biomedical science. Biomedicine is a valuable guide for the readers to become aware of current advancement in rapidly moving field of biomedicine.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Wei Xia and Jing Ni (2012). RNA Interference for Tumor Therapy, Biomedicine, Dr. Chao Lin (Ed.), ISBN: 978-953-51-0352-3, InTech, Available from: <http://www.intechopen.com/books/biomedicine/sirna-interference-for-cancer-therapy>

**INTech**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.